In re Application of: Michal AMIT et al.
Serial No.: 10/537,784
Filed: 06/06/2005
Office Action Mailing Date: 01/28/2008

Examiner: Deborah Crouch
Group Art Unit: 1632
Attorney Docket: 29606

In the claims:

1-152 (Cancelled).

153. (Currently amended) A method of establishing a feeder cells-free human embryonic stem cell line which is ~~capable of being~~ maintained in an undifferentiated, pluripotent and proliferative state, the method comprising:

(a) obtaining stem cells of a human ~~embryonic stem cells~~ embryo, and;

(b) culturing said stem cells of said human embryo~~nic stem cells~~ under culturing conditions devoid of feeder cells and including a matrix and a tissue culture medium supplemented with TGFβ_1 and ~~,~~ bFGF ~~and/or LIF~~ to thereby obtain the feeder cells-free human embryonic stem cell line.

154. (Previously presented) The method of claim 153, further comprising cloning a cell from the human embryonic stem cell line resultant from step (b) under said culturing conditions.

155. (Currently amended) A method of propagating a human embryonic stem cell line in an undifferentiated, pluripotent and proliferative state under culturing conditions devoid of feeder cells, the method comprising culturing cells of the human embryonic stem cell line on a matrix and a tissue culture medium which comprises ~~supplemented with~~ TGFβ_1 and~~,~~ bFGF ~~and/or LIF~~ to thereby maintain the cells of the human embryonic stem cell line in an undifferentiated, pluripotent and proliferative state.

156. (Previously presented) The method of claim 153, wherein said matrix is a fibronectin matrix.

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157. (Previously presented) The method of claim 156, wherein said fibronectin is selected from the group consisting of bovine fibronectin, recombinant bovine fibronectin, human fibronectin, recombinant human fibronectin, mouse fibronectin, recombinant mouse fibronectin, and synthetic fibronectin.

158. (Currently amended) The method of claim 153, wherein said culturing conditions are substantially free of xeno contaminant and where ~~i~~as said matrix is selected from the group consisting of human plasma fibronectin matrix, recombinant human plasma fibronectin matrix, human cellular fibronectin matrix, recombinant human cellular fibronectin matrix, synthetic fibronectin.

159. (Previously presented) The method of claim 153, wherein the human embryonic stem cell line comprises at least 85 % of undifferentiated human embryonic stem cells.

160. (Previously presented) The method of claim 153, wherein the cells of the human embryonic stem cell line maintain a doubling time of at least 25 hours.

161. (Currently amended) The method of claim 153, wherein said tissue culture medium further ~~includes~~ comprises serum and/or serum replacement.

162. (Previously presented) The method of claim 161, wherein said serum and/or said serum replacement is provided at a concentration of at least 10 %.

163. (Previously presented) The method of claim 161, wherein said serum and/or said serum replacement is provided at a concentration of 15 %.

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164. (Previously presented) The method of claim 153, wherein said $TGF\beta_1$ is provided at a concentration of at least 0.06 ng/ml.

165. (Previously presented) The method of claim 153, wherein said $TGF\beta_1$ is provided at a concentration of 0.12 ng/ml.

166. (Previously presented) The method of claim 153, wherein said bFGF is provided at a concentration of at least 2 ng/ml.

167. (Previously presented) The method of claim 153, wherein said bFGF is provided at a concentration of 4 ng/ml.

168. (Currently amended) The method of claim 153, wherein said tissue culture medium further comprises LIF. ~~is provided at a concentration of at least 500 u/ml.~~

169. (Currently amended) The method of claim ~~153~~168, wherein said LIF is provided at a concentration of 1000 u/ml.

170. (Currently amended) A method of establishing a xeno – free, feeder cells-free embryonic stem cell line of a species which is ~~capable of being~~ maintained in an undifferentiated, pluripotent and proliferative state, the method comprising:

(a) obtaining ~~embryonic~~ stem cells of an embryo of the species, and;

(b) culturing said ~~embryonic~~ stem cells under culturing conditions devoid of feeder cells and xeno contaminants and including a species - derived matrix devoid of xeno contaminants and a tissue culture medium devoid of xeno contaminants, said tissue culture medium comprises TGFβ1 and bFGF, to thereby obtain the xeno – free, feeder cells-free embryonic stem cell line of the species.

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171. (Currently amended) A method of propagating a species embryonic stem cell line in an undifferentiated, pluripotent and proliferative state under culturing conditions devoid of feeder cells and xeno contaminants, the method comprising culturing cells of the species embryonic stem cell line on a species - derived matrix devoid of xeno contaminants and a tissue culture medium devoid of xeno contaminants, said tissue culture medium comprises TGFβ1 and bFGF, to thereby maintain the cells of the species embryonic stem cell line in an undifferentiated, pluripotent and proliferative state.

172. (Previously presented) The method of claim 170, wherein said matrix is a species – derived fibronectin matrix.

173. (Previously presented) The method of claim 170, wherein said feeder cells-free culturing conditions are substantially free of xeno contaminants.

174. (Previously presented) The method of claim 170, wherein the species embryonic stem cell line comprises at least 85 % of undifferentiated species embryonic stem cells.

175. (Previously presented) The method of claim 170, wherein the cells of the species embryonic stem cell line maintain a doubling time of at least 20 hours.

176. (Currently amended) The method of claim 170, wherein said tissue culture medium further comprises ~~includes~~ a species - derived serum and/or a serum replacement.

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177. (Previously presented) The method of claim 176, wherein said species - derived serum is provided at a concentration of at least 5 %.

178. (Previously presented) The method of claim 176, wherein said serum replacement is provided at a concentration of at least 10 %.

179. (Previously presented) The method of claim 176, wherein said serum replacement is provided at a concentration of 15 %.

180. (Cancelled)

181. (Currently amended) The method of claim ~~180~~171, wherein said tissue culture medium further comprises ~~at least one growth factor is selected from the group consisting of TGFβ_1, bFGF, and~~ LIF.

182. (Currently amended) The method of claim ~~181~~171, wherein said $TGF\beta_1$ is provided at a concentration of at least 0.06 ng/ml.

183. (Currently amended) The method of claim ~~181~~171, wherein said $TGF\beta_1$ is provided at a concentration of 0.12 ng/ml.

184. (Currently amended) The method of claim ~~181~~171, wherein said bFGF is provided at a concentration of at least 2 ng/ml.

185. (Currently amended) The method claim ~~181~~171, wherein said bFGF is provided at a concentration of 4 ng/ml.

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186. (Previously presented) The method of claim 181, wherein said LIF is provided at a concentration of at least 500 u/ml.

187. (Previously presented) The method of claim 181, wherein said LIF is provided at a concentration of 1000 u/ml.

188. (Currently amended) A method of establishing a xeno – free, feeder cells-free embryonic stem cell line of a species which is maintained in an undifferentiated, pluripotent and proliferative state, the method comprising:

(a) obtaining stem cells of an embryo of the species, and;

(b) culturing said stem cells under xeno-free culturing conditions devoid of feeder cells and xeno contaminants and including a species - derived matrix and a ~~The method of any of claim 170, wherein said tissue culture medium is a~~ species – derived conditioned medium, to thereby obtain the xeno – free, feeder cells-free embryonic stem cell line of the species.

189. (Currently amended) A cell culture comprising undifferentiated, pluripotent and proliferative human embryonic stem cells in a culture medium, said culture medium comprising TGFβ1 and bFGF, wherein the cell culture is substantially free of xeno- and/~~or~~ feeder cells contaminants.

190. (Currently amended) The cell culture of claim 189, wherein the culture medium further comprises ~~includes~~ serum replacement.

191. (Previously presented) The cell culture of claim 190, wherein said serum replacement is provided at a concentration of at least 10 %.

192. (Previously presented) The cell culture of claim 190, wherein said serum replacement is provided at a concentration of 15 %.

193. (Currently amended) The cell culture of claim ~~190~~189, wherein said culture medium further comprises ~~includes TGFβ$_1$, bFGF and/or~~ LIF.

194. (Currently amended) The cell culture of claim ~~193~~ 189, wherein said TGFβ$_1$ is provided at a concentration of at least 0.06 ng/ml.

195. (Currently amended) The cell culture of claim 189 ~~193~~, wherein said TGFβ$_1$ is provided at a concentration of 0.12 ng/ml.

196. (Currently amended) The cell culture of claim 189 ~~193~~, wherein said bFGF is provided at a concentration of at least 2 ng/ml.

197. (Currently amended) The cell culture of claim 189 ~~193~~, wherein said bFGF is provided at a concentration of 4 ng/ml.

198. (Previously presented) The cell culture of claim 193, wherein said LIF is provided at a concentration of at least 500 u/ml.

199. (Previously presented) The cell culture of claim 193, wherein said LIF is provided at a concentration of 1000 u/ml.

200. (Previously presented) The cell culture of claim 189, wherein said human embryonic stem cells are maintainable in an undifferentiated, pluripotent and proliferative state for at least 38 passages.

201. (Previously presented) The cell culture of claim 189, wherein said human embryonic stem cells maintain a doubling time of at least 25 hours.

202. (Previously presented) The cell culture of claim 189, wherein said human embryonic stem cells comprise at least 85 % of undifferentiated stem cells.

203. (Currently amended) A xeno-free, feeder cells-free culture system comprising a matrix devoid of xeno contaminants and a tissue culture medium devoid of xeno contaminants, said culture medium comprises TGFβ1 and bFGF, the xeno-free, feeder cells-free culture system ~~being selected capable of~~ maintains ~~ing~~ human embryonic stem cells cultured therein in a proliferative, pluripotent and undifferentiated state.

204. (Previously presented) The culture system of claim 203, wherein said matrix is human-derived fibronectin.

205. (Previously presented) The culture system of claim 204, wherein said human-derived fibronectin is selected from the group consisting of human plasma fibronectin, recombinant human plasma fibronectin, human cellular fibronectin, recombinant human cellular fibronectin, and synthetic fibronectin.

206. (Currently amended) The culture system of claim 203, wherein said tissue culture medium further comprises ~~includes~~ serum replacement.

207. (Previously presented) The culture system of claim 206, wherein said serum replacement is provided at a concentration of at least 10 %.

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208. (Previously presented) The culture system of claim 206, wherein said serum replacement is provided at a concentration of 15 %.

209. (Currently amended) The culture system of claim 203, wherein said tissue culture medium further comprises ~~includes TGFβ1, bFGF and/or~~ LIF.

210. (Currently amended) The culture system of claim ~~209~~ 203, wherein said $TGF\beta_1$ is provided at a concentration of at least 0.06 ng/ml.

211. (Currently amended) The culture system of claim 203 ~~209~~, wherein said $TGF\beta_1$ is provided at a concentration of 0.12 ng/ml.

212. (Currently amended) The culture system of claim 203 ~~209~~, wherein said bFGF is provided at a concentration of at least 2 ng/ml.

213. (Currently amended) The culture system of claim 203 ~~209~~, wherein said bFGF is provided at a concentration of 4 ng/ml.

214. (Previously presented) The culture system of claim 209, wherein said LIF is provided at a concentration of at least 500 u/ml.

215. (Previously presented) The culture system of claim 209, wherein said LIF is provided at a concentration of 1000 u/ml.

216. (Previously presented) The culture system of claim 203, wherein said human embryonic stem cells comprise at least 85 % of undifferentiated human embryonic stem cells.

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217. (Previously presented) The culture system of claim 203, wherein said human embryonic stem cells maintain a doubling time of at least 25 hours.

218. (Withdrawn) A method of treating an individual in need of cell replacement and/or tissue regeneration, comprising administering a human embryonic stem cell preparation being free of xeno and feeder cells contaminants to the individual.

219. (Withdrawn and currently amended) The method of claim 218, further comprising preparing said human embryonic stem cell preparation prior to said administering, said preparing being effected by:

(a) obtaining human embryonic stem cells, and;

(b) culturing said human embryonic stem cells under culturing conditions devoid of feeder cells and xeno contaminants and including a human-derived fibronectin matrix and a tissue culture medium supplemented with $TGF\beta_1$, and bFGF ~~and/or LIF~~ to thereby prepare the human embryonic stem cell preparation.

220. (Withdrawn) The method of claim 219, wherein said human-derived fibronectin is selected from the group consisting of human plasma fibronectin, recombinant human plasma fibronectin, human cellular fibronectin, recombinant human cellular fibronectin, and synthetic fibronectin.

221. (Currently amended) A method of maintaining human embryonic stem cells in an undifferentiated, pluripotent and proliferative state under culturing conditions devoid of feeder cells, the method comprising culturing the human embryonic stem cells under culturing conditions including a matrix and a tissue culture medium, said culture medium comprises TGFβ1 and bFGF ~~supplemented with at least one growth factor~~ provided at a concentration range which ~~selected capable of~~

maintains ~~ing~~ said stem cells for at least 56 passages with a doubling time of at least 25 hours.

222. (Previously presented) The method of claim 221, wherein said human embryonic stem cells comprise at least 85 % of undifferentiated human embryonic stem cells.

223. (Previously presented) The method of claim 221, wherein said matrix is selected from the group consisting of human-derived fibronectin, human-derived laminin, foreskin fibroblast matrix, MEFs matrix.

224. (Previously presented) The method of claim 223, wherein said human-derived fibronectin is selected from the group consisting of human plasma fibronectin, recombinant human plasma fibronectin, human cellular fibronectin, recombinant human cellular fibronectin, and synthetic fibronectin.

225. (Currently amended) The method of claim 221, wherein said tissue culture medium further comprises ~~at least one growth factor is selected from the group consisting of TGFβ_1, bFGF, and~~ LIF.

226. (Currently amended) The method of claim ~~225~~ 221, wherein said TGFβ_1 is provided at a concentration range of 0.06-0.24 ng/ml.

227. (Currently amended) The method of claim ~~225~~ 221, wherein said bFGF is provided at a concentration range of 2-8 ng/ml.

228. (Currently amended) The method of claim ~~226~~225, wherein said LIF is provided at a concentration range of 500-2000 u/ml.

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229. (Currently amended) A method of maintaining human embryonic stem cells in an undifferentiated, pluripotent and proliferative state under culturing conditions devoid of feeder cells, the method comprising culturing the human embryonic stem cells under culturing conditions including an extracellular matrix and tissue culture medium ~~The method of claim 221, wherein said culturing conditions~~ which includes serum replacement at a concentration of 15 %, $TGF\beta_1$ at a concentration of 0.12 ng/ml, LIF at a concentration of 1000 u/ml, and bFGF at a concentration of 4 ng/ml.

230. (New) The method of claim 168, wherein said LIF is provided at a concentration of at least 500 u/ml.

231. (New) The method of claim 155, wherein said $TGF\beta_1$ is provided at a concentration of at least 0.06 ng/ml.

232. (New) The method of claim 155, wherein said $TGF\beta_1$ is provided at a concentration of 0.12 ng/ml.

233. (New) The method of claim 155, wherein said bFGF is provided at a concentration of at least 2 ng/ml.

234. (New) The method of claim 155, wherein said bFGF is provided at a concentration of 4 ng/ml.

235. (New) The method of claim 155, wherein said tissue culture medium is further supplemented with LIF.

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236. (New) The method of claim 235, wherein said LIF is provided at a concentration of at least 500 u/ml.

237. (New) The method of claim 235, wherein said LIF is provided at a concentration of at least 1000 u/ml.